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A Histochemical Study of Glycosidases in Benign Prostatic Hyperplasia and in Prostatic Carcinoma in the Human

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Summary. In benign hyperplasia marked β-glucuronidase and N-acetyl-β-glucosaminidase activity was seen in the prostatic epithelium whereas the reactions for 5 other glycosidases were only slight or negative. The intensity of the reaction for the glycosidases in prostatic carcinoma was dependent upon the degree of the differentiation. The possible importance of glycosidases for the invasiveness of prostatic carcinoma is briefly discussed.

Key words: Prostatic adenoma - Prostatic carcinoma - Glycosidases.

In the western world prostatic carcinoma is one of the most frequent types of cancer in men after the age of 50. After cancer of the lung and stomach it is the third most frequent of all malignant neoplasms in this age group (15).

An important measurement in the diagnosis of prostatic carcinoma is the determination of serum phosphatases. The diagnosis of prostatic carcinoma may be considered almost definite where there is a significant increase in tartrate labile acid phosphatase.

It is therefore surprising that the histochemical activity of acid phosphatase in prostatic carcinoma tissue is less than in normal prostate (1, 5, 10). The increase in serum phosphatase levels could be explained either by the presence of metastases producing acid phosphatase or by the loss of polarity in the prostatic cells as a result of malignant dedifferentiation. Thus, contrary to normal, the acid phosphatase is not secreted into the lumina of the acini but passes into the surrounding stroma and enters the bloodstream (10).

Distinct changes in activity in other hydrolases, such as leucylamino-peptidase and β-glucuronidase can also be observed in prostatic carcinoma (5, 10). Whereas a reduction in the activity of leucylamino-peptidase can be observed in the very early stages of prostatic carcinoma, β-glucuronidase exhibits increased activity in prostatic carcinoma, as in other malignant tumours (5, 10).

β-glucuronidase is a member of the glycosidase group for which high values in the male accessory sex organs have been determined by biochemical methods (2). The prerequisite for a more detailed interpretation of the function of these enzymes is their exact histological localisation. The aim of this paper is to carry out comparative histochemical examinations of the glycosidases in benign prostatic hyperplasia and prostatic carcinoma in order to determine the alterations in enzyme pattern which may occur in malignant tumours.

MATERIAL AND METHODS

5 prostatic adenomas and 9 histologically proven prostatic carcinomas were examined. The material was obtained from suprapubic

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Table 1

Enzyme	Substrate	Reference
N-Acetyl-β-glucosaminidase (β-Nag)	Naphthol-AS-BI-N-Acetyl-β-glucosaminide	Hayashi, (7)
β-Glucuronidase (β-Glu)	Naphthol-AS-BI-glucuronide	Hayashi, (6)
β-Galactosidase (β-Gal)	6-Brom-2-Naphthyl-β-galactopyranoside	Rutenburg et al., (13)
β-Fucosidase (β-Fuc)	4-Cl-5Br-3-Indolyl--β-D-Fucoside	Lojda und Kraml (11)
α-Mannosidase (α-Man)	1-Naphthyl-α-mannopyranoside	Gossrau, (3)
α-Galactosidase (α-Gal)	1-Naphthyl-α-galactopyranoside	Gossrau, (3)
α-Fucosidase (α-Fuc)	1-Naphthyl-α-L-fucopyranoside	Gossrau, (4)

Specificity controls: Incubation of sections without substrate and of sections inactivated by heat treatment.
Additional inhibition tests: Inhibition of α-mannosidase with 100 mM mannonolactone (3) and of β-glucuronidase with 100 mM saccharolactone (3).

prostatectomies and transurethral electroresections. The samples were shock-frozen in liquid nitrogen immediately after removal. The presence of the various glycosidases listed in Table 1 was investigated on 10 µm thick cryostat sections. The references for the methods used are detailed in Table 1.

RESULTS

1. Benign Prostatic Hyperplasia (Prostatic Adenoma)

On histochemical examination of N-Acetyl-β-glucosaminidase (β-Nag) a distinct granular reaction occurred in the high-columnar epithelium which lines the majority of the glandular acini, this reaction being slightly greater in the basal parts of the epithelial cells than in the remainder of the cytoplasm. The nuclei of the high-columnar cells situated in the basal third of the epithelium and the nuclei of the basal cells in the immediate neighbourhood of the basal membrane showed no reaction. Definite β-Nag activity was also observed in the cytoplasm of the flat isoprismatic epithelial cells of the enlarged cystic acini which occur in varying numbers amongst those appearing normal. Material with distinct β-Nag activity was present in the lumina of many acini. In the proliferated connective tissue the intensity of enzyme reaction was only weak. However, strongly β-Nag positive cells occurred sporadically in the stroma and these were probably macrophages.

A similar pattern of activity and distribution was also seen with β-Glucuronidase (β-Glu). The epithelium of the acini regularly showed a distinct granular reaction, β-Glu positive secretions could be detected in the lumina and the proliferated connective tissue again showed only a weakly positive reaction.

The β-Galactosidase (β-Gal) test showed a reaction in the glandular epithelium which was in general weak and diffuse. Only the supranuclear cell area showed a distinctly positive reaction. The large β-Gal positive vacuoles occurring at the base of some acini are conspicuous. The reaction of the secretions in the glandular lumina was fairly positive. The β-Gal activity in the interstitium was weak.

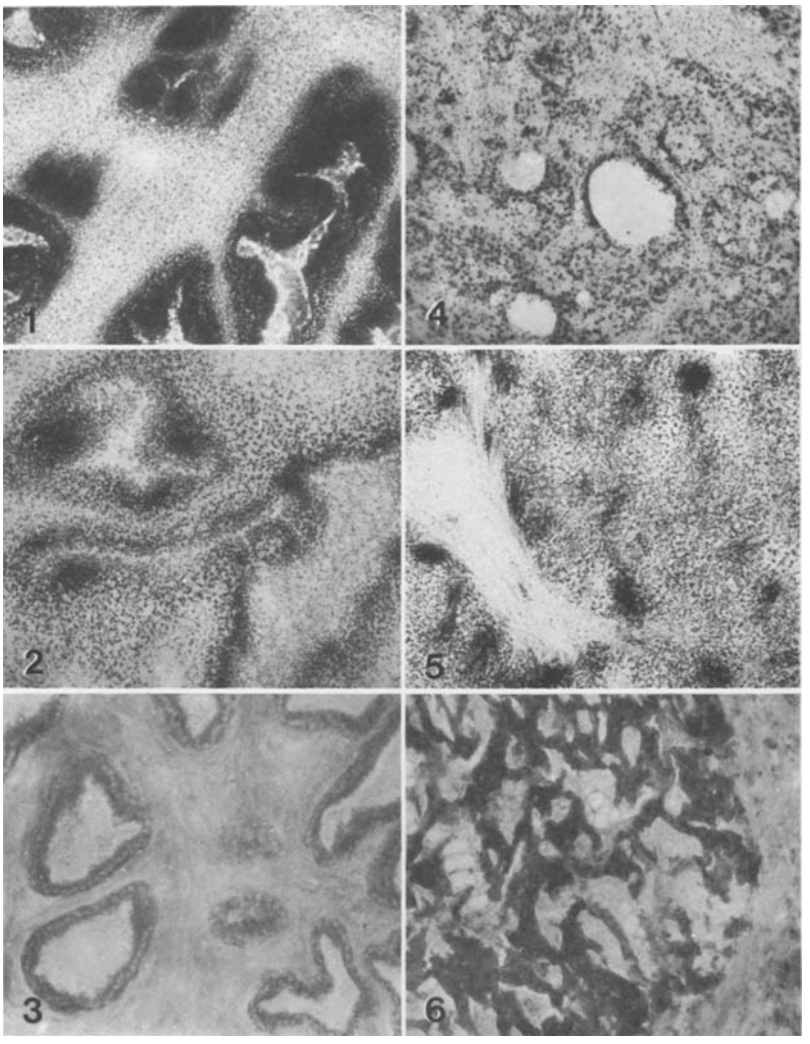

Fig. 1. Prostatic adenoma, N-acetyl-β-glucosaminidase, x 127.5

Fig. 2. Prostatic adenoma, β-glucuronidase, x 127.5

Fig. 3. Prostatic adenoma, β-galactosidase, x 127.5

Fig. 4. Prostatic adenocarcinoma, β-glucuronidase, x 127.5

Fig. 5. Solid carcinoma of the prostate, N-acetyl-β-glucosaminidase, x 127.5

Fig. 6. Anaplastic carcinoma of the prostate, N-acetyl-β-glucosaminidase, x 127.5

In the β-Fucosidase (β-Fuc) test only sporadic enzyme positive granules could be seen in the epithelium of the acini.

The other glycosidases could not be detected in prostatic adenomatous tissue.

2. Prostatic Carcinoma

The prostatic carcinomas were classified according to Kastendieck and Altenähr (9) into

a) adenocarcinoma
b) solid and/or cribriform carcinoma
c) anaplastic carcinoma

Adenocarcinoma: In the β-Nag and β-Glu tests the epithelial cells of the generally small acini in the well-differentiated areas showed a distinct granular reaction and there was weak reaction to the β-Gal test. β-Fuc reaction was also weak and in some cases could not be detected by histochemical methods. In the stroma between the glandular acini a weak reaction was observed for β-Nag and β-Glu.

Solid/Cribriform Carcinomas: In the compact cell aggregates of solid prostatic carcinomas only moderate granular reactions to the β-Glu and β-Nag enzyme tests were seen in large areas of the tumours. These findings are in striking contrast to the intense reaction observed in isolated cell groups lying between these tumour cells. In the solid/cribriform carcinoma a consistently weak reaction for β-Gal was observed, without the occurrence of any strong reaction in isolated cell groups. In the β-Fuc test, isolated enzyme positive granules occurred in the cytoplasm of the tumour cells.

Anaplastic Carcinomas: In the cytoplasm of the tumour cells which penetrate between the stroma in the form of narrow cell cords high β-Nag and β-Glu activity was demonstrated.

The β-Gal test was also highly positive in some cases. The β-Fuc activity was weak. As in the case of the adenocarcinomas and solid/cribriform cancers it was impossible to detect any reaction for the other glycosidases tested.

In the β-Nag and β-Glu tests there was a distinct reaction in the degenerating stroma in the areas adjacent to the tumour cells.

DISCUSSION

In the histochemical β-Glu and β-Nag-tests it was possible to observe a distinct granular reaction in the epithelium of prostatic acini of patients with prostatic adenomas. The presence of numerous granular reaction products in the entire epithelium indicates that both enzymes are localized in the lysosomes and numerous secretory vacuoles of the well-differentiated epithelium cells. The activity and distribution of both glycosidases are therefore very similar to the conditions in the normal prostate (14). These findings and also the occurrence of enzyme positive matter in the glandular lumina indicate that the behaviour of secretory processes in prostatic adenomas is normal.

In prostatic cancer the enzyme pattern was very different. We were not able to observe a general overall increase in the activity of β-Glu, as claimed by Györkey (5). The intensity of the reaction was, indeed, distinctly dependent upon the degree of differentiation and upon the invasiveness of the individual tumours. The pattern of activity for well-differentiated adenocarcinomas was similar to that of the prostatic adenoma. In the case of solid/cribriform cancers we observed greatly differing reactions within the tumour areas. Cell groups showing extraordinarily strong reaction in the β-Glu and β-Nag-tests protrude from amongst the mass of tumour cells reacting more weakly. These cell groups are probably tumour cells with particularly high proliferative ability.

In anaplastic cancers high activity of β-Glu and β-Nag was seen in the cell cords infiltrating the stroma in all cases. In the tests for these two glycosidases a distinct granular reaction was also frequently observed in the adjacent degenerating stroma. It is possible that β-Glu and β-Nag are of particular importance for the invasiveness of anaplastic prostatic cancers. Glycosidases are active in the metabolism of connective tissue ground substance (12). We suggest that due to the release of glycosidases from the anaplastic tumour cells there is depolymerisation of the ground substance. This proposition is supported by the electron microscope examinations carried out by Kastendieck and Altenähr (8, 9). They found that anaplastic tumour cells carcinomas lost the polarity of normal prostatic cells and that the vacuoles, which under normal circumstances secrete into the glandular lumen, were particularly numerous in the cytoplasm adjacent to the stroma. The damage to the ground substance caused by the release of glycosidases from the tumour cells could lead to impairment of the metabolism of the stroma cells and to their ensuing degeneration. The lysosomal enzymes released by the degenerating connective tissue cells may further accelerate the degradation of the ground substance and facilitate the invasion of cancer cells.

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REFERENCES

1. Brandes, D., Bourne, G. H.: Histochemistry of some aspects of prostate pathology. Biology of the prostate and related tissues. National Cancer Institute Monographs 12, 29-91 (1963)
2. Conchie, J., Findlay, J., Levvy, G. A.: Mammalian glycosidases distribution in the body. Biochemical Journal 711, 318 (1959)
3. Gossrau, R.: Über den histochemischen Nachweis der β-Glucuronidase, α-Mannosidase und α-Galactosidase mit 1-Naphthylglykosiden. Histochemie 36, 367 (1973)
4. Gossrau, R.: Über die Eignung von Naphthyl- α-L-Fucosiden zur Untersuchung der α-L-Fucosidasen. Histochemistry 52, 259 (1977)
5. Györkey, F.: Some Aspects of Cancer of the Prostate Gland. Methods in Cancer Research. Vol. 10, Ed. H. Busch. New York: Academic Press 1973
6. Hayashi, M., Nakajima, Y., Fishman, W. H.: The cytoplasmic demonstration of β-Glucuronidase employing Naphthol-AS-BI-Glucosaminide and Hexazonium Pararosanilin; a preliminary report. Journal of Histochemistry and Cytochemistry 12, 293 (1964)
7. Hayashi, M.: Histochemical demonstration of N-Acetyl-β-Glucosaminidase employing Naphthol-AS-BI-N-Acetyl-β-Glucosaminid as substrate. Journal of Histochemistry and Cytochemistry 13, 355 (1965)

8. Kastendieck, H., Altenähr, E., Burkhardt, P.: Elektronenmikroskopische Untersuchungen zur Zelldifferenzierung in Prostata-Carcinomen. Virchows Archiv; A: Pathological Anatomy and Histology 361, 241 (1973)
9. Kastendieck, H., Altenähr, E.: Cyto- and histomorphogenesis of the prostate carcinome. Virchows Archiv; A: Pathological Anatomy and Histology 370, 207 (1976)
10. Kirchheim, D., Brandes, D., Bacon, R. L.: Fine Structure and Cytochemistry of Human Prostatic Carcinoma. Male Accessory Sex Organs. Ed. by D. Brandes. New York: Academic Press 1974
11. Lojda, Z., Kraml, J.: Indigogenic methods for Glycosidases III. An inproved method with 4-Cl-5Br-3-Intolyl-β-D-Fucoside and its application in studies of enzymes in the intestine, kidney and other tissues. Histochemie 25, 195 (1977)
12. Riede, U. N., Staubesand, J.: A unifying concept for the role of matrix vesicles and lysosomes in the formal pathogenesis of diseases of connective tissues and blood vessels. Beiträge zur Pathologie 160, 3 (1977)
13. Rutenburg, A. M., Rutenburg, S. M., Mones, B., Teaque, R., Seligman, A. M.: Histochemical demonstration of β-D-Galactosidase in the rat. Journal of Histochemistry and Cytochemistry 6, 122 (1958)
14. Sinowatz, F., Weber, P., Skolek-Winnisch, R., Mossig, H.: Histotopik von Glykosidasen in der Prostata des Menschen. (In preparation)
15. Wynder, E. L., Mabuchi, K., Whitmore, W. F.: Epidemiology of cancer of the prostate. Cancer 28, 344 (1971)

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